

## INVESTIGATION OF FUNGAL METABOLITES AND ACUTE TOXICITY STUDIES FROM FRUIT-BODIES OF *HYPHOLOMA* SPECIES (*STROPHARIACEAE*)

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**ABSTRACT.** - The fruit-bodies of *Hypholoma fasciculare* and *H. capnoides* were screened by thin-layer chromatography to detect polyols, sugars, phenolic acids, alkaloids and fungal metabolites. Mannitol, trehalose, 4-hydroxybenzoic acid, vanillic acid and choline were mainly observed from both *Hypholoma* species. The fungal toxins,  $\alpha$ -amanitin, bufotenine, muscarine, muscimol and orellanine were not detected. Acute toxicity studies and toxicological investigations were carried out in mice given single intraperitoneal doses of suspensions of the methanol extracts from *H. fasciculare* and *H. capnoides*. Paralysis of the respiratory centre, severe stomachal ulcers and digestive hemorrhages were revealed with *H. fasciculare* (LD50 value: 243.29 mg/kg) while no pathology was observed with *H. capnoides*.

**RÉSUMÉ.** - Une étude chimique des métabolites primaires et secondaires a été réalisée sur *Hypholoma fasciculare* et *H. capnoides* par chromatographie sur couche mince. Le mannitol, le tréhalose, l'acide parahydroxybenzoïque, l'acide vanillique et la choline ont été mis en évidence chez les deux espèces. Par contre, les toxines fongiques,  $\alpha$ -amanitine, bufoténine, muscarine, muscimol et orellanine n'ont pas été observées. Des études toxicologiques ont été réalisées chez la souris par injection intrapéritonéale des extraits méthanoliques de *H. fasciculare* et de *H. capnoides*. Une paralysie du centre respiratoire, de sévères ulcères stomacaux et des hémorragies digestives ont été décrits chez *H. fasciculare* (DL50 : 243, 29 mg/kg). Aucune pathologie n'a observée pour *H. capnoides*.

**Key-words:** *Hypholoma capnoides*; *Hypholoma fasciculare*; LD50; phenolic acids; polyols; sugars.

## INTRODUCTION

The poisonous bitter tasting mushroom *H. fasciculare* (Huds. : Fr.) Kumm. and the edible-like mild species *H. capnoides* (Fr. : Fr.) Kumm. have a worldwide distribution (Bon, 1988). Chemical studies and fatal poisonings were only reported for *H. fasciculare* (Wasiljkow, 1963; Herrmann, 1967; Ondrusek and Prostenik, 1984; Badalyan and Nikichenko, 1991). According to other authors, this species caused gastrointestinal troubles and vomiting (Kachkin *et al.*, 1979; Bresinsky and Besl, 1990), and had fibrinolytic and hemolytic activities (Badalyan, 1993). Lanostane-type triterpenes were identified as toxic principles for *H. fasciculare* causing paralysis and death (Suzuki *et al.*, 1983; Kubo *et al.*, 1985). No toxicological study was described on *H. capnoides*.

In this work, methanolic and aqueous extracts of *H. fasciculare* and *H. capnoides* were investigated for polyols, sugars, phenolic acids, alkaloids and fungal metabolites using thin-layer chromatography methods (Badalyan *et al.*, 1994). Results of acute toxicity studies and toxicological investigations in the mice exposed to *H. fasciculare* and *H. capnoides* were also reported.

## MATERIALS AND METHODS

### 1. Material

*H. fasciculare* and *H. capnoides* were collected at Regensburg in Bavaria (October 1993). The species were preserved by drying after morphological identification from fresh materials. Chemical analyses were performed on a homogeneous fine powder.

### 2. Methods

#### Preparation of extracts

The methanolic and aqueous extractions from fruit-bodies of *H. fasciculare* and *H. capnoides* were performed according to the method of Badalyan *et al.* (1994).

Aliquots of the residues from the methanolic extract were used for thin-layer chromatography (TLC) analyses of polyols, sugars, alkaloids and fungal metabolites, and also resuspended in 3% aqueous carboxymethyl cellulose for toxicological investigations.

Aliquots of the residues from the aqueous extract were used for TLC analyses of polyols, sugars and phenolic acids.

Concentration and R<sub>f</sub> value of standards relative to TLC analyses were previously described (Rapior *et al.*, 1990; Badalyan *et al.*, 1994).

#### Qualitative estimation of polyols and sugars

Acyclic polyols and free sugars from the methanolic and aqueous extracts of *H. fasciculare* and *H. capnoides* were analyzed by TLC according to Andary *et al.* (1979) and Rapior *et al.* (1990).

### Qualitative determination of phenolic acids

The phenolic acids were extracted and analyzed by two-dimensional TLC (Rapior *et al.*, 1990; Badalyan *et al.*, 1994).

### Qualitative detection of fungal metabolites

The methanolic extracts from *H. fasciculare* and *H. capnoides* were analyzed by TLC developed with methanol-water-acetic acid (90:5:5, v/v) after spraying with Dragendorff's reagent modified according to Bregoff-Delwiche for alkaloids and quaternary ammonium (Stahl, 1969), and sulfanilic acid diazotised for  $\alpha$ -amanitin, bufotenine and muscimol (Andary *et al.*, 1977).

The methanolic extracts from *H. fasciculare* and *H. capnoides* were also analyzed for orellanine by TLC according to Rapior *et al.* (1988).

### Acute toxicity studies and toxicological investigations

Adult, male and female SWISS mice (from 5 to 6 weeks old) weighing 24-26 g and 16-19 g, respectively, were used for these experiments. The animals were housed 3 male and 5 female per cage (25 x 45 x 15 cm), respectively. The room temperature was 22°C and relative humidity 60  $\pm$  10 %. Artificial light was the only source of light and the animals were set on a 12 hour light/dark cycle. The mice had free access to commercial pelleted diet and tap water. They were intraperitoneally (i.p.) injected with suspensions of the methanolic or aqueous extract residues from *H. fasciculare* and the methanolic extract residue from *H. capnoides* in 3% aqueous carboxymethyl cellulose. All suspensions were prepared in such a manner that 10 ml was given per kg of body weight. LD50 (i.p.) values were calculated by the method of Behrens and Karber (1935).

Single i.p. doses of the methanolic extract from *H. fasciculare* were given to eight groups of 3 male and eight groups of 5 female (2000, 1000, 500, 250, 200, 150, 100, 50 mg/kg). Five groups of 3 male and five groups of 5 female were treated with suspensions of the aqueous extract from *H. fasciculare* as follows: 1000, 500, 250, 150, 100 mg/kg. Varying single doses of the methanolic extract from *H. capnoides* were administered to four groups of 3 male and four groups of 5 female (2000, 1000, 500, 250 mg/kg).

Toxicological evaluations (tests of platform, traction, loss of dynamic activity and catalepsy; flexion, posture, pineal, corneal and Haffner reflexes) were carried out at 30 min, each hour from 1 to 6 h, on day 5, 10 and 14. The animals were weighed on day 1, 5, 10 and 14. Survivors were killed two weeks after dosing in the acute toxicity studies.

## RESULTS AND DISCUSSION

### 1. Polyol and sugar contents

No arabitol or galactose was detected in *H. fasciculare* and *H. capnoides*. Both species contained mannitol and trehalose. Furthermore, the presence of glucose and fructose was detected in *H. fasciculare* and *H. capnoides*, respectively.

## 2. Phenolic acid content

Chromatographic examination of the aqueous extract from *Hypholoma* species demonstrated the presence of mono- and diphenolic acids. Two monophenolic acids, 4-hydroxybenzoic acid and 4-hydroxy 3-methoxybenzoic acid (= vanillic acid) were observed in *H. fasciculare* and *H. capnoides*. 3,4-dihydroxyphenylacetic acid and 4-hydroxycinnamic acid (= coumaric acid) were detected in *H. fasciculare* and *H. capnoides*, respectively.

## 3. Fungal metabolite contents

Choline was identified from *H. fasciculare* and *H. capnoides*. The presence of this compound had not been reported in *Hypholoma* and proved to be of no significance chemotaxonomically as also reported for *Cortinarius* (Tebbett et al., 1983; Badalyan et al., 1994) and *Rhodophyllus* (Maki et al., 1985). On the other hand, we did not find any trace of betaine and the fungal toxins,  $\alpha$ -amanitin, bufotenine, muscarine, muscimol and orellanine in *H. fasciculare* and *H. capnoides*.

## 4. Acute toxicity studies and toxicological investigations

### *H. fasciculare.*

The single i.p. LD50 dose for the methanolic extract of *H. fasciculare* was 243.29 mg/kg in mice. On the other hand, the aqueous extract obtained after the methanolic extraction of *H. fasciculare* was not toxic up to 1000 mg/kg.

At 2000 mg/kg dose from the methanolic extract, tremors appeared after 20 minutes and the animals died from paralysis of the respiratory centre. At doses ranging from 1000 to 250 mg/kg, symptoms of toxicity included subdued behavior with depression, sensation of coldness and loss of muscle coordination. The mice died from severe ulcers and internal digestive hemorrhages within 5 days after administration.

All the animals were prostrated with total muscular relaxation at doses ranging from 2000 to 500 mg/kg. Mice given single i.p. 2000 or 1000 mg/kg doses revealed reduction of pineal and flexion reflexes, and troubles of autonomic nervous system with breathing disturbances. One mice out of 3 treated with 500 mg/kg dose had no grasp reflex while 2 of 3 had an uncertain reflex due to muscular slackening. Reduction of mobility was observed for 66% of mice at 250 mg/kg dose. Catalepsy test, and posture and corneal reflexes were normal, whatever doses i.p. administered. No lacrymation or mydriasis was observed.

### *H. capnoides.*

No death or pathology was seen in the mice i.p. injected with suspensions of the methanolic extract of *H. capnoides* at doses ranging from 2000 mg/kg to 250 mg/kg. This was confirmed by the animal weight at different doses for the same period of time. Autopsy of animals sacrificed on day 14, revealed no digestive or pulmonary changes.

## CONCLUSION

Acyclic polyols, free sugars and phenolic acids are widely distributed within the fungi. The occurrence of these fungal metabolites can contribute useful information for the classification of mushrooms (Andary *et al.*, 1979; 1988; Rast and Pfyffer, 1989; Rapior *et al.*, 1990).

TLC carried out on *H. fasciculare* and *H. capnoides* suggest that their sugar and phenolic acid contents are not similar. Our results are then in good agreement with those of other authors who consider these two taxa to be chemotaxonomically different (Gluchoff-Fiasson and Kühner, 1977; De Bernardi *et al.*, 1981).

On the other hand, acute toxicity studies and toxicological investigations confirmed that *H. fasciculare* is lethal for SWISS mice (LD50: 243.29 mg/kg). Paralysis of the respiratory centre and severe digestive hemorrhages caused the mice to die when given i.p. doses.

Thus, our toxicological investigations supported the opinion that *H. capnoides* was non toxic and could be considered as an edible-like mushroom. However, interpretation of edibility should be done carefully for this species.

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